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(57) Abstract

The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

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9. PRO4357

Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many of these efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. We herein describe the identification and characterization of novel secreted polypeptides, designated herein as PRO4357 polypeptides.

10. PRO4405

Efforts are being undertaken by both industry and academia to identify new, native transmembrane receptor proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel transmembrane receptor proteins. We herein describe the identification and characterization of novel transmembrane polypeptides, designated herein as PRO4405 polypeptides.

11. PRO4356

Glycosylphosphatidylinositol (GPI) anchored proteoglycans are generally localized to the cell surface and are thus known to be involved in the regulation of responses of cells to numerous growth factors, cell adhesion molecules and extracellular matrix components. The metastasis-associated GPI-anchored protein (MAGPIAP) is one of these cell surface proteins which appears to be involved in metastasis. Metastasis is the form of cancer wherein the transformed or malignant cells are traveling and spreading the cancer from one site to another. Therefore, identifying the polypeptides related to metastasis and MAGPIAP is of interest.

12. PRO4352

Cadherins are a large family of transmembrane proteins. Cadherins comprise a family of calcium-dependent glycoproteins that function in mediating cell-cell adhesion in virtually all solid tissues of multicellular organisms. At least cadherins 1-13 as well as types B, E, EP, M, N, P and R have been characterized. Among the functions cadherins are known for, with some exceptions, cadherins participate in cell aggregation and are associated with cell-cell adhesion sites. Recently, it has been reported that while all cadherins share multiple repeats of a cadherin specific motif believed to correspond to folding of extracellular domains, members of the cadherin superfamily have divergent structures and, possibly, functions. In particular it has been reported that members of the cadherin superfamily are involved in signal transduction. See, Suzuki, J. Cell Biochem., 61(4):531-542 (1996). Cadherins are further described in Tanihara, et al., J. Cell Sci., 107(6):1697-1704 (1994), Aberle, et al., J. Cell Biochem., 61(4):514-523 (1996), Obata, et al., Cell Adhes. Commun., 6(4):323-33 (1998) and Tanihara, et al., Cell Adhes. Commun., 2(1):15-26 (1994).

Protocadherins are members of the cadherin superfamily which are highly expressed in the brain. In some studies, protocadherins have shown cell adhesion activity. See, Sano, et al., EMBO J., 12(6):2249-2256 (1993). However, studies have also shown that some protocadherins, such as protocadherin 3 (also referred to as Pcdh3 or pc3), do not show strong calcium dependent cell aggregation activity. See, Sago, et al., Genomics,

Another embodiment is directed to fragments of a PRO4356 polypeptide coding sequence that may find use as hybridization probes. Such nucleic acid fragments are from about 20 through about 80 nucleotides in length, preferably from about 20 through about 60 nucleotides in length, more preferably from about 20 through about 50 nucleotides in length, and most preferably from about 20 through about 40 nucleotides in length.

In another embodiment, the invention provides isolated PRO4356 polypeptide encoded by any of the isolated nucleic acid sequences hereinabove defined.

In a specific aspect, the invention provides isolated native sequence PRO4356 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 20 through 251 of Figure 22 (SEQ ID NO:50).

In another aspect, the invention concerns an isolated PRO4356 polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 20 to about 251, inclusive of Figure 22 (SEQ ID NO:50).

In a further aspect, the invention concerns an isolated PRO4356 polypeptide, comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 20 through 251 of Figure 22 (SEQ ID NO:50).

In yet another aspect, the invention concerns an isolated PRO4356 polypeptide, comprising the sequence of amino acid residues 20 to about 251, inclusive of Figure 22 (SEQ ID NO:50), or a fragment thereof sufficient to provide a binding site for an anti-PRO4356 antibody. Preferably, the PRO4356 fragment retains a qualitative biological activity of a native PRO4356 polypeptide.

In a still further aspect, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO4356 polypeptide having the sequence of amino acid residues from about 20 to about 251, inclusive of Figure 22 (SEQ ID NO:50), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

12. <u>PRO4352</u>

A cDNA clone (DNA87976-2593) has been identified that encodes a novel polypeptide having homology to protocadherin pc3 designated in the present application as "PRO4352".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO4352 polypeptide.

In one aspect, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO4352 polypeptide having

the sequence of amino acid residues from 1 or about 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52), or (b) the complement of the DNA molecule of (a).

In another aspect, the invention concerns an isolated nucleic acid molecule encoding a PRO4352 polypeptide comprising DNA hybridizing to the complement of the nucleic acid between about residues 257 and about 2578, inclusive, of Figure 23 (SEQ ID NO:51). Preferably, hybridization occurs under stringent hybridization and wash conditions.

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 203888 (DNA87976-2593), or (b) the complement of the DNA molecule of (a). In a preferred embodiment, the nucleic acid comprises a DNA encoding the same mature polypeptide encoded by the human protein cDNA in ATCC Deposit No. 203888 (DNA87976-2593).

In a still further aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues from about 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52), or the complement of the DNA of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule having at least about 50 nucleotides, and preferably at least about 100 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO4352 polypeptide having the sequence of amino acid residues from about 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52), or (b) the complement of the DNA molecule of (a), and, if the DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), isolating the test DNA molecule.

In a specific aspect, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO4352 polypeptide, with or without the N-terminal signal sequence and/or the initiating methionine, and its soluble variants (i.e. transmembrane domain deleted or inactivated), or is complementary to such encoding nucleic acid molecule. The signal peptide has been tentatively identified as extending from amino acid position 1 through about amino acid position 26 in the sequence of Figure 24 (SEQ ID NO:52). The transmembrane domain has been tentatively identified as extending from about amino acid position 687 through about amino acid position 711 in the PRO4352 amino acid sequence (Figure 24, SEQ ID NO:52).

In another aspect, the invention concerns an isolated nucleic acid molecule comprising (a) DNA encoding a polypeptide scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52), or (b) the complement of the DNA of (a).

Another embodiment is directed to fragments of a PRO4352 polypeptide coding sequence that may find use as hybridization probes. Such nucleic acid fragments are from about 20 through about 80 nucleotides in length, preferably from about 20 through about 60 nucleotides in length, more preferably from about 20 through about 50 nucleotides in length, and most preferably from about 20 through about 40 nucleotides in length.

In another embodiment, the invention provides isolated PRO4352 polypeptide encoded by any of the isolated nucleic acid sequences hereinabove defined.

In a specific aspect, the invention provides isolated native sequence PRO4352 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 27 through 800 of Figure 24 (SEQ ID NO:52).

In another aspect, the invention concerns an isolated PRO4352 polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to the sequence of amino acid residues 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52).

In a further aspect, the invention concerns an isolated PRO4352 polypeptide, comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 27 through 800 of Figure 24 (SEQ ID NO:52).

In yet another aspect, the invention concerns an isolated PRO4352 polypeptide, comprising the sequence of amino acid residues 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52), or a fragment thereof sufficient to provide a binding site for an anti-PRO4352 antibody. Preferably, the PRO4352 fragment retains a qualitative biological activity of a native PRO4352 polypeptide.

In a still further aspect, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO4352 polypeptide having the sequence of amino acid residues from about 27 to about 800, inclusive of Figure 24 (SEQ ID NO:52), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

13. <u>PRO4380</u>

A cDNA clone (DNA92234-2602) has been identified that encodes a novel polypeptide designated in the present application as "PRO4380".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO4380 polypeptide.

In one aspect, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO4380 polypeptide having

Figure 14 shows the amino acid sequence (SEQ ID NO:29) derived from the coding sequence of SEQ ID NO:28 shown in Figure 13.

Figure 15 shows a nucleotide sequence (SEQ ID NO:34) of a native sequence PRO4353 cDNA, wherein SEQ ID NO:34 is a clone designated herein as "DNA80145-2594".

Figure 16 shows the amino acid sequence (SEQ ID NO:35) derived from the coding sequence of SEQ ID NO:34 shown in Figure 15.

Figure 17 shows a nucleotide sequence (SEQ ID NO:39) of a native sequence PRO4357 cDNA, wherein SEQ ID NO:39 is a clone designated herein as "DNA84917-2597".

Figure 18 shows the amino acid sequence (SEQ ID NO:40) derived from the coding sequence of SEQ ID NO:39 shown in Figure 17.

Figure 19 shows a nucleotide sequence (SEQ ID NO:44) of a native sequence PRO4405 cDNA, wherein SEQ ID NO:44 is a clone designated herein as "DNA84920-2614".

Figure 20 shows the amino acid sequence (SEQ ID NO:45) derived from the coding sequence of SEQ ID NO:44 shown in Figure 19.

Figure 21 shows a nucleotide sequence (SEQ ID NO:49) of a native sequence PRO4356 cDNA, wherein SEQ ID NO:49 is a clone designated herein as "DNA86576-2595".

Figure 22 shows the amino acid sequence (SEQ ID NO:50) derived from the coding sequence of SEQ ID NO:49 shown in Figure 21.

Figure 23 shows a nucleotide sequence (SEQ ID NO:51) of a native sequence PRO4352 cDNA, wherein SEQ ID NO:51 is a clone designated herein as "DNA87976-2593".

Figure 24 shows the amino acid sequence (SEQ ID NO:52) derived from the coding sequence of SEQ ID NO:51 shown in Figure 23.

Figure 25 shows a nucleotide sequence (SEQ ID NO:56) of a native sequence PRO4380 cDNA, wherein SEQ ID NO:56 is a clone designated herein as "DNA92234-2602".

Figure 26 shows the amino acid sequence (SEQ ID NO:57) derived from the coding sequence of SEQ ID NO:56 shown in Figure 25.

Figure 27 shows a nucleotide sequence (SEQ ID NO:58) of a native sequence PRO4354 cDNA, wherein SEQ ID NO:58 is a clone designated herein as "DNA92256-2596".

Figure 28 shows the amino acid sequence (SEQ ID NO:59) derived from the coding sequence of SEQ ID NO:58 shown in Figure 27.

Figure 29 shows a nucleotide sequence (SEQ ID NO:60) of a native sequence PRO4408 cDNA, wherein SEQ ID NO:60 is a clone designated herein as "DNA92274-2617".

Figure 30 shows the amino acid sequence (SEQ ID NO:61) derived from the coding sequence of SEQ ID NO:60 shown in Figure 29.

Figure 31 shows a nucleotide sequence (SEQ ID NO:62) of a native sequence PRO5737 cDNA, wherein SEQ ID NO:62 is a clone designated herein as "DNA92929-2534".

Figure 32 shows the amino acid sequence (SEQ ID NO:63) derived from the coding sequence of SEQ ID NO:62 shown in Figure 31.

terminus.

10. **Full-length PRO4405 Polypeptides**

As far as is known, the DNA84920-2614 sequence encodes a novel factor designated herein as PRO4405; using WU-BLAST2 sequence alignment computer programs, limited sequence identities to known proteins were revealed.

11. **Full-length PRO4356 Polypeptides**

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO4356 (shown in Figure 22 and SEQ ID NO:50) has certain amino acid sequence identity with metastasis associated GPI-anchored protein. Accordingly, it is presently believed that PRO4356 disclosed in the present application is a newly identified member of this family and shares similar mechanisms.

12. **Full-length PRO4352 Polypeptides**

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO4352 (shown in Figure 24 and SEQ ID NO:52) has certain amino acid sequence identity with protocadherin pc3 and protocadherin pc4. Accordingly, it is believed that PRO4352 is involved in cell adhesin and can be used in treatments regarding differentiation disorders, cell adhesin, neural receptor or skin disorders. Moreover, it can be used in screens to identify agonists and antagonists to treat such disorders.

13. **Full-length PRO4380 Polypeptides**

As far as is known, the DNA92234-2602 sequence encodes a novel factor designated herein as PRO4380; using WU-BLAST2 sequence alignment computer programs, limited sequence identities to proteins with known functions were revealed.

14. **Full-length PRO4354 Polypeptides**

As far as is known, the DNA92256-2596 sequence encodes a novel factor designated herein as PRO4354; using WU-BLAST2 sequence alignment computer programs, limited sequence identities to proteins with known functions were revealed.

15. **Full-length PRO4408 Polypeptides**

As far as is known, the DNA92274-2617 sequence encodes a novel factor designated herein as PRO4408; using WU-BLAST2 sequence alignment computer programs, limited sequence identities to known proteins were revealed.

16. **Full-length PRO5737 Polypeptides**

Using WU-BLAST2 sequence alignment computer programs, it has been found that a full-length native sequence PRO5737 (shown in Figure 32 and SEQ ID NO:63) has certain amino acid sequence identity with IL-1

EXAMPLE 14: Isolation of cDNA clones Encoding Human PRO4356

A consensus DNA sequence was assembled relative to other EST sequences using phrap asdescribed in Example 1 above. This consensus sequence is designated herein "DNA80200". Based upon an observed homology between the DNA80200 consensus sequence and an EST sequence contained within Merck EST clone 248287, Merck EST clone 248287 was purchased and its insert obtained and sequenced, thereby providing DNA86576-2595.

The entire coding sequence of PRO4356 is shown in Figure 21 (SEQ ID NO:49). Clone DNA86576-2595 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 55-57, and an apparent stop codon at nucleotide positions 808-810. The predicted polypeptide precursor is 251 amino acids long. Clone DNA86576-2595 has been deposited with ATCC and is assigned ATCC deposit no. 203868. The full-length PRO4356 protein shown in Figure 22 has an estimated molecular weight of about 26,935 daltons and a pI of about 7.42.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 22 (SEQ ID NO:50), revealed homology between the PRO4356 amino acid sequence and the following Dayhoff sequences incorporated herein: RNMAGPIAN_1, UPAR_BOVIN, S42152, AF007789_1, UPAR_RAT, UPAR_MOUSE, P_W31165, P_W31168, P_R44423 and P_W26359.

EXAMPLE 15: Isolation of cDNA clones Encoding Human PRO4352

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is designated herein "DNA83397". Based on the DNA83397 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO4352.

PCR primers (forward and reverse) were synthesized:

forward PCR primer: 5'-CTGGGGAGTGTCCTTGGCAGGTTC-3' (SEQ ID NO:53) and

reverse PCR primer: 5'-CAGCATACAGGGCTCTTTAGGGCACAC-3' (SEQ ID NO:54).

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA83397 sequence which had the following nucleotide sequence:

hybridization probe: 5'-CGGTGACTGAGGAAACAGAGAAAGGATCCTTTGTGGTCAATCTGGC-3' (SEQ ID NO:55).

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO4352 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal brain.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO4352 (designated herein as DNA87976-2593 [Figure 23, SEQ ID NO:51]; and the derived protein sequence for PRO4352.

The entire coding sequence of PRO4352 is shown in Figure 23 (SEQ ID NO:51). Clone DNA87976-2593 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 179-181, and an apparent stop codon at nucleotide positions 2579-2581 of SEQ ID NO:51. The predicted polypeptide precursor is 800 amino acids long. Clone DNA87976-2593 has been deposited with ATCC and is assigned ATCC deposit no. 203888. The full-length PRO4352 protein shown in Figure 24 has an estimated molecular weight of about 87,621 daltons and a pI of about 4.77.

An analysis of the Dayhoff database (version 35:45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 24 (SEQ ID NO:52), revealed homology between the PRO4352 amino acid sequence and the following Dayhoff sequences: P_R86865, P_R86866, RATPCDH_1, AB011160_1, MMU88549_1, D86917_1, AB008179_1, P_R58907, HSHFATPRO_1, and AF031572_1.

EXAMPLE 16: Isolation of cDNA clones Encoding Human PRO4380

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster sequence from the Incyte database. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altshul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA79132. In light of DNA79132, DNA92234-2602 was identified.

The full length clone shown in Figure 25 contained a single open reading frame with an apparent translational initiation site at nucleotide positions 201-203 and ending at the stop codon found at nucleotide positions 1722-1724 (Figure 25; SEQ ID NO:56). The predicted polypeptide precursor (Figure 26, SEQ ID NO:57) is 507 amino acids long. PRO4380 has a calculated molecular weight of approximately 56,692 daltons and an estimated pI of approximately 5.22.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 26 (SEQ ID NO:57), revealed homology between the PRO4380 amino acid sequence and the following Dayhoff sequences (sequences and related text incorporated herein): CER11H6_1, S56299, D89150_1, G70870, S43914, LMO34616_5, LLU78036_1, AF055904_2, P_W79066 and ARGE_ECOLI.

Clone DNA92234-2602 was deposited with the ATCC and is assigned ATCC deposit no. 203948.

EXAMPLE 17: Isolation of cDNA clones Encoding Human PRO4354

Use of the signal sequence algorithm described in Example 3 above allowed identification of an EST cluster (92909) sequence designated herein as DNA10195. This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing

Ethanolamine, 100μl of 10^{-1} M (Sigma E0135)

Phosphoethalamine, 100μl of 10^{-1} M (Sigma P0503)

Selenium, 4μl of 10^{-1} M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

Hydrocortisone, 2μl of $5X10^{-3}$ M (Sigma #H0135)

Progesterone, 100μl of $1X10^{-3}$ M (Sigma #P6149)

Forskolin, 500μl of 20mM (Calbiochem #344270)

Minimal media:

RPMI 1640 plus transferrin (10 μg/ml), insulin (1 μg/ml), gentamycin (100 ng/ml), aprotinin (50 μg/ml) and BPE (15 μg/ml).

Defined media:

RPMI 1640 plus transferrin (10 μg/ml), insulin (1 μg/ml), gentamycin (100 ng/ml) and aprotinin (50 μg/ml).

The following polypeptides were positive in this assay: PRO4356.

EXAMPLE 40: Fetal Hemoglobin Induction in an Erythroblastic Cell Line (Assay 107)

This assay is useful for screening PRO polypeptides for the ability to induce the switch from adult hemoglobin to fetal hemoglobin in an erythroblastic cell line. Molecules testing positive in this assay are expected to be useful for therapeutically treating various mammalian hemoglobin-associated disorders such as the various thalassemias. The assay is performed as follows. Erythroblastic cells are plated in standard growth medium at 1000 cells/well in a 96 well format. PRO polypeptides are added to the growth medium at a concentration of 0.2% or 2% and the cells are incubated for 5 days at 37°C. As a positive control, cells are treated with 100μM hemin and as a negative control, the cells are untreated. After 5 days, cell lysates are prepared and analyzed for the expression of gamma globin (a fetal marker). A positive in the assay is a gamma globin level at least 2-fold above the negative control.

The following polypeptides tested positive in this assay: PRO4352, PRO4354, PRO4408, PRO6030 and PRO4499.

EXAMPLE 41: Mouse Kidney Mesangial Cell Proliferation Assay (Assay 92)

This assay shows that certain polypeptides of the invention act to induce proliferation of mammalian kidney mesangial cells and, therefore, are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with Schönlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease. The assay is performed as follows. On day one, mouse kidney mesangial cells are plated on a 96 well plate in growth media (3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 95% fetal bovine serum, 5% supplemented with 14 mM HEPES) and grown overnight. On day 2, PRO polypeptides are diluted at 2 concentrations(1% and 0.1%) in serum-free medium and added to the cells. Control samples are serum-free medium alone. On day 4, 20μl of the Cell Titer 96 Aqueous one solution reagent (Progema) was added to each well and the colormetric reaction was allowed